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An affordable solution for investigating zebra finch intracranial EEG signals V.1

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We use this protocol and it's working

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Abstract

The Zebra finch is a well-studied model animal for neural mechanisms of speech, and Electrophysiology is the primary technique for understanding the song system in them. Most of the studies on zebra finches have focused on intracerebral recordings. However, these methods are not readily available and expensive. Recently, different open-source hardware for acquiring EEG signals has been developed. It's unclear if these solutions are suitable zebra finches as they haven't been evaluated yet. Electrocorticography signals, which have been overlooked in avian electrophysiology, can be helpful in studying bird songs. They can provide a preliminary guide for more in-depth inquiries and also aid in understanding the global behavior of the bird's brain, as opposed to the more common localized approach. We provide a detailed protocol for acquiring ECOG data from zebra finches with an open-source device (open BCI). We implemented stainless steel electrodes on the brain's surface and recorded the brain signals from two recording sites. To validate our method, we ran two different experiments. In the first experiment, we recorded neural activity under different concentrations of isoflurane and extracted the burst suppression rate (BSR) to measure anesthesia depth. In the second experiment, we head-fixed the birds and presented them with different auditory stimuli to evaluate event-related potential. Results showed a significant increase in the BRS by increasing the anesthesia depth. And significant ERP response to auditory stimuli. These findings indicate that by our methodology, we can successfully collect ECOG signals from awake and anesthetized birds.



Materials

stainless steel electrodes

isoflurane

open bci bord

meloxicam

ceftriaxone

lidocaine

betadine

surgical blade

dental cement

insulin syringe

connectors

needle for head post

stereotaxic device

anesthesia mask

bench vice

raspberry bi 3



Hardware preparation

1 Electrode preparation:

30m

For recording electrodes, we used 2.5 cm of stainless steel wire  150 \$ and uncoat two ends of it with a scalpel blade. make a loop in one end about 1 or 2mm in diameter. use a surgical clamp to bend it. ***Do not make a bigger loop, it may not fit into the hole in the skull.*** check the connection by multimeter

2 Printing and assembling the anesthesia mask:

1d

We designed a 3d printable adaptor for gas delivery and head fixation. It consists of 2 parts. Before surgery, fix the adaptor and check the fixation step and gas leakage. PLA printed materials cost around  20 \$

 base.stl 828.5KB funnel.stl

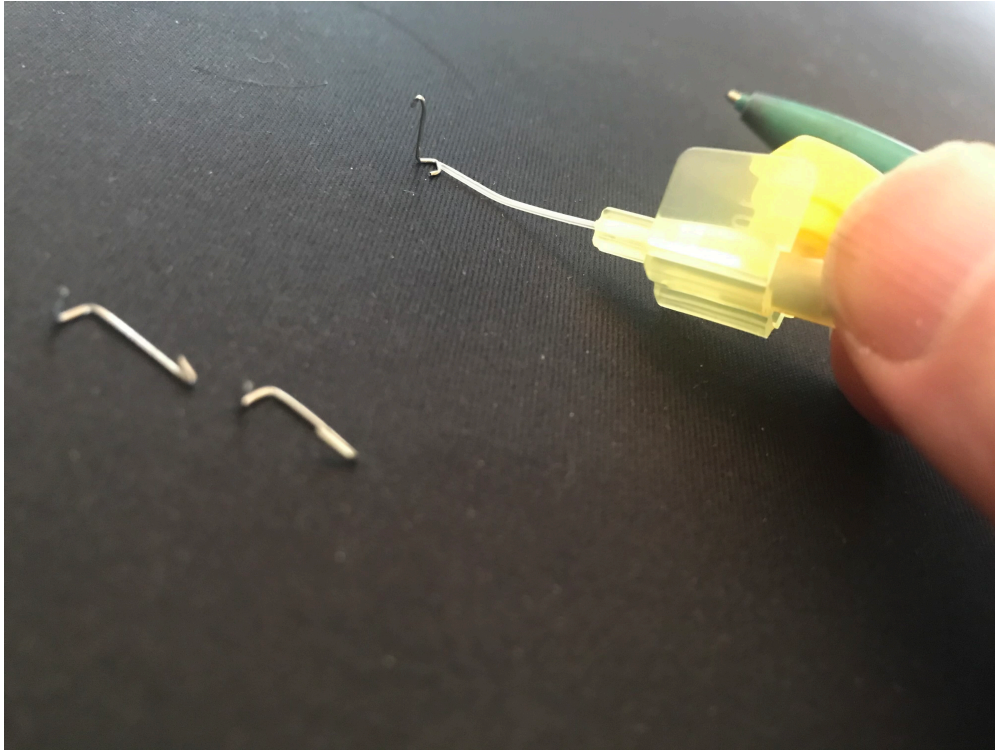
3 Building ear bar adaptor:

We used a hypodermic needle base after covering the tip of it with silicon to avoid ear trauma. Check the length of the bar adaptors. They should be equal for reaching the precise fixation.

4 Building headpost:

10m

To create the head post, use a hypodermic needle and shape it with a hemostat clamp. blunt sharp edges of the needle.



head posts

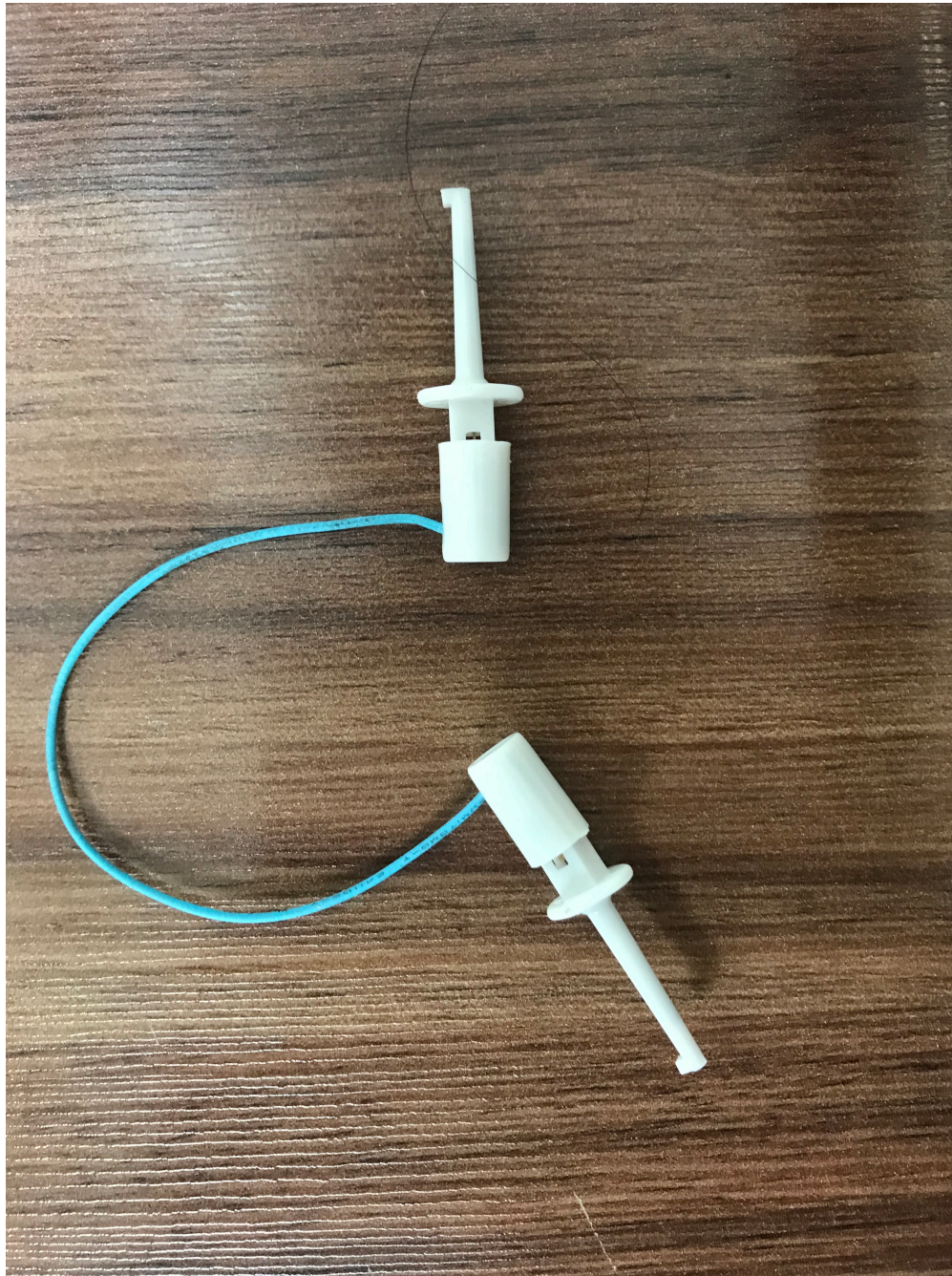
5 Electrode and head post disinfection:

Reserve all the electrodes inside a 70 percent alcohol solution until you need to use them

6 Preparation and testing connectors:

Connectors are two oscilloscope clamps soldered to the two ends of a wire. Prepare 4 connectors and check their connectivity by multimeter

1h



Electrode to bci board connector

7 Setup recording hardware and software:

Make sure the device has enough battery power check batteries with a multimeter. You can do it according to the [OpenBCI getting started](https://openbci.org/getting-started) on its website.

3h

preoperative procedures

50m



8 **Animal:**
female or male zebra finches  15 g and older than 60 days is suitable for this operation. Avoid using underweighted animals because of the lower survival rate through anesthesia

9 **Prepare animal for surgery:**
Restrict the food intake for  00:30:00 prior to operation, and restrict water consumption  00:10:00 before the surgery. Do not restrict feeding to more than 30 minutes if you exceed this time chose normal saline with dextrose for the first fluid injection to prevent hypoglycemia.

40m

10 **Prepare drugs:**
Calculate the dosage of drugs and load the syringes before the procedure to avoid wasting time and mistakes during surgery. Label all loaded syringes clearly. Use insulin syringes and a subcutaneous route of administration.
ringer lactate
lidocaine
Diluted Povidone-iodine
clindamycin
ciprofloxacin (eye drop)
dental cement

15m



11 **Autoclave the surgery equipment:**  00:10:00
Scalpel Handle number 3
Tampons
Swaps
Surgical drill

10m

Surgery

10m

12 **Anesthesia induction:**
Place the animal inside the induction chamber.
Start with 2 minutes of oxygen delivery and then increase the gas concentration by 0.5 percent every 1 minute. Continue increasing the concentration until you reach 2 percent. Keep the animal under the gas chamber for two minutes.
inject ringer lactate every Duration 00:20:00 throughout the entire operation.

Safety information

Isoflurane leakage from anesthesia machines can affect experimenters' health. To avoid this issue, use a suitable scavenging system. Staff should be well informed and educated about the possible hazards **of anesthetic gas**.

13 Head fixation:

Quickly insert the animal's beak into the gas cylinder and monitor the depth of anesthesia. If the depth decreases, check for any sources of leakage. You may need to increase the flow rate, as the narrow passage of the beak holder will increase resistance. Wait until the animal reaches a stable condition before proceeding. Place the animal's ear between ear bars and firmly fix the head and gently open its beak. Insert the maxilla into the beak holder, push the beak holder bar toward the bird's head, and rotate the screw until the beak is held tightly in place.



14 Preparation of the incision site and skull for craniotomy:

Pick the feather of the skull gently to remove all of the covering feathers of the head. We use the scalp not only as an incision site but also as the subcutaneous injection site to disinfect the surface of the skull by applying the diluted betadine and inject  10 μL

of diluted lidocaine, wait for  00:05:00 for the drug to be absorbed

Make a midline incision from the point between the eyes to the cerebellum. Retract the edges of the incision. The bifurcation site should be immediately recognizable. Scrape the skull to remove the periosteum using a tiny bite of a sterilized tampon. Allow the skull to dry for  00:05:00

10m

15 Craniotomy:

"to avoid neural damage due to frictional heating, drilling sites should be hydrated continuously with normal saline."

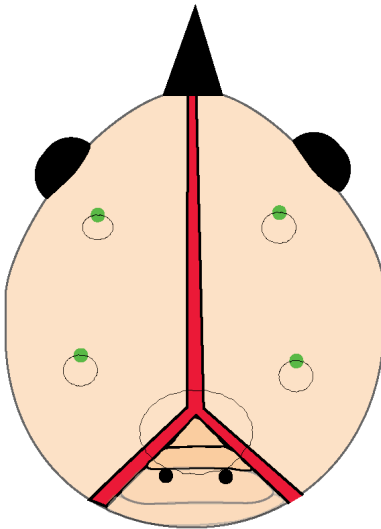
Locate the bifurcation site beneath the skull. If the skull is dried and opaque, rehydrate the bone with a saline drop to find the bifurcation site. Drill the skull around the bifurcation site in a  2.5 mm diameter and remove the detached bone with a

hemostat clamp. The cerebellum is evident in the removed circle, and you can use the bifurcation site as a pivot of your coordinate coordination for recording sites as follows:

recording site 1 = AP:  1 mm , ML:  2.2 mm

recording site 2 = AP :  5 mm , ML:  2 mm

Locate the recording site on the skull and drill a hole to reach the dura.



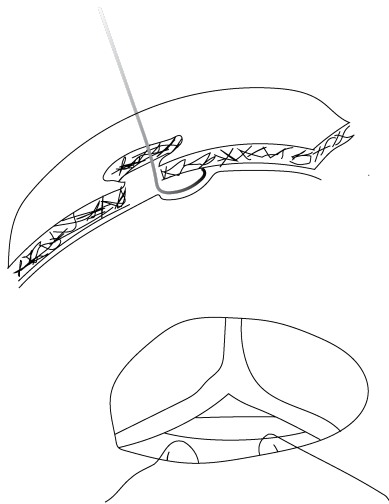
The recording site is depicted in green, and bias and ground electrodes are depicted (We used two sites for recording)

16 Electrode placement:

'preserve electrode inside ethanol solution. Make sure that the alcohol is completely evaporated before implanting the electrode.'

Place the noise canceling and the grand electrode on the surface of the cerebellum, as indicated in the picture. Insert the loop part of the electrodes in recording sites between the skull and dura matter. The upward force of the brain tissue will fix the electrode in place.





17 **Cementing the skull:**

prepare the skull for cement adhesion and apply local antibiotics drill the surface skull to make the surface rough and advisable for dental cement apply one drop of ciprofloxacin eye drop on the skull and try to spread by cotton swab to fill the holes let the antibiotic to penetrate to all parts of the skull

18 **Implanting head post**

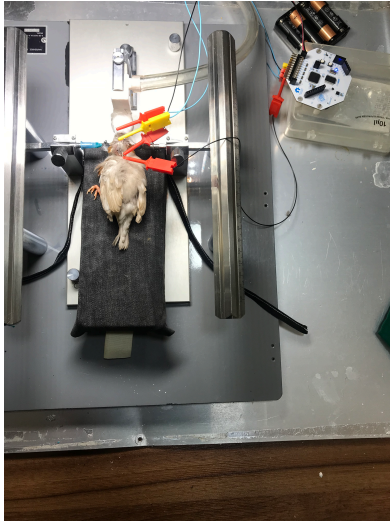
Place **the base of** the headpost on the forming cement When it has a creamy consistency add more cement to it and hold the head post perpendicularly to the skull until the cement formed. you can test the hardness of the cement by tapping with a needle.

19 wait until the cement cured completely

Recording EEG first experiment

20 **Connecting the electrodes:**

Connect the noise and grand electrodes to the electrodes on the cerebellum and use two recording channels



connected electrodes to the open bci board in anesthetized bird

21 **Check the signal:**

Immediately after connecting the electrodes, one should see the pattern of the activities characteristic of the isoflurane anesthesia



22 **Recording EEG from anesthetized animal:**

start recording at 0.5 concentration and proceed at 0.5 percent every two minutes until reaching 2 percent. Reverse the order of gas after reaching concentration 2 to decrease



the

Recovery

23

make sure that the cement is completely cured

24

inject meloxicam and clindamycin

25

Animal housing :

avoid placing birds in multiple cages they try to cut the electrodes of their friends. place their cages close to each other to avoid the stress of social isolation.

Recovery

26

Animal housing :

do not keep the birds in the same cage. They try to pick the electrodes of their cagemates.

Place their cages close to each other to minimize the effect of social isolation stress.

Awake EEG setup

27

Adaptation period :

during  168:00:00 adaptation period monitor the bird to detect any adverse effects. usually, birds adapt well to implanted materials during this period. some adverse neurological problems may occur like movement disorders, and hearing or vision loss. check the animals to exclude them in case these symptoms are presented. ask veterinary aid to diagnose these possible problems

1w

28

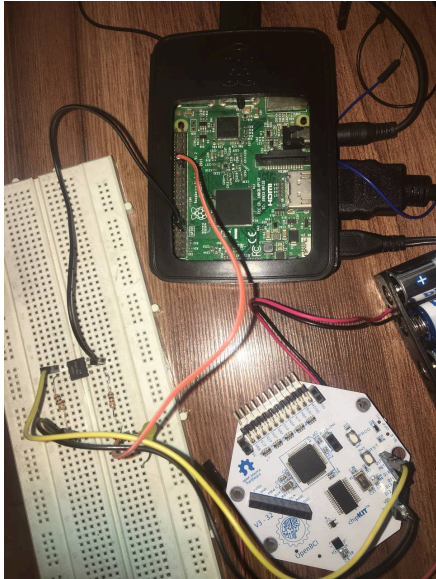
Trigger system:

For delivering the external signal to the open bci board input pins on the board can be used. however, the external trigger signal source should be isolated to avoid electrical noise contamination of the signal this is done by an optocoupler circuit.

29

Stimulus presenting :

We used Raspberry Pi 3  34.99 \$ for presenting auditory stimuli and sending trigger signals to the open bci



trigger system including raspberry pi and optcoupler circuit

30

Animal fixation apparatus :

we used a mini bench vice and a surgery hemostat clamp for fixating the head of the bird in one position. The body was embraced by a trimmed 20 ml string cylinder.restraining

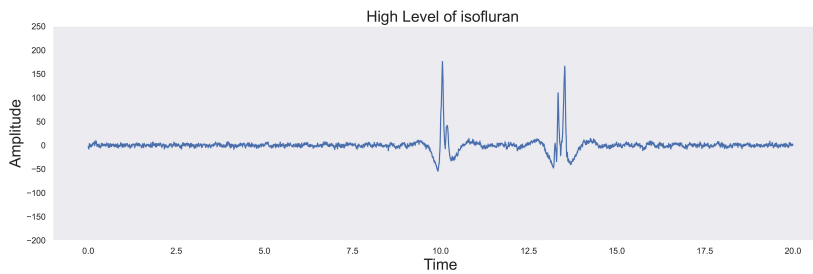


head fixed bird inside restraining chamber with connected electrodes

Method and analysis

31 **Burst suppression:**

We used burst suppression as an index for anesthesia depth



signals during deep anesthesia, burst, and suppression periods are visually detectable

All codes are available in a git hub repository.

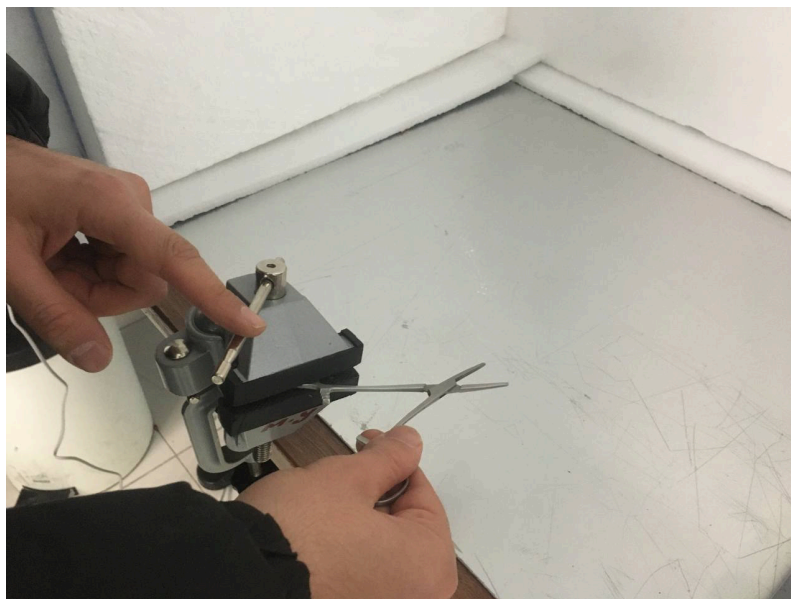
https://github.com/Miladyekani/zebrfa_finch_EEG.git

Awake EEG data acquisition

32 **Adjusting the clamp position Animal fixation:**

Tighten one of the finger holes of the hemostat forceps between the jaws of the bench vice. Adjust the position of the vice to locate the tip of the forceps in a place where you want to fix the head post

. Lock the head post between the jaws of the forceps, restrain the bird with your hands to avoid struggling, and then ask your assistant to put the bird's body inside the tube and make sure the bird's head is in the right position. Attach the electrodes and measure the impedance.



Adjusting the clamp position

33 **Stimuli presentation:**

open the open bci GUI, close the acoustic chamber, and then start the stimuli-presenting program. monitor the triggers arrivals on the GUI. We used pin 17 as inputs in the board if the



Acoustic recording chamber